A NEW PUPAL AND IMAGINAL ABERRATION OF THE MARSH FRITILLARY *EURODRYAS AURINIA* ROTT. (LEP.: NYMPHALIDAE)

K. E. J. Bailey

Dipfield, Thorverton, nr Exeter, Devon, EX5 5PJ.

A breeding culture of *Eurodryas aurinia* was established in March 1991 by collecting a few feral post-hibernation larvae from several areas within a widespread colony in Devonshire, S. W. England. It is probable that these originated from at least three wild females. The aim of this culture was to produce successive generations for cold-shock experiments on young pupae under various regimes and to select, if possible, for post-cold-shock expression of ab. *sebaldus* Schultz phenotypes. Since the original experiments by Standfuss (1900) on *Aglais urticae* L., the author has found that moderate cold shock does not induce sterility in *E. aurinia*, *Argynnis paphia* L. and *Polygonia c-album* L.

Breeding technique

During the early stages, including hibernation, the young larvae were reared in tubs on growing devil's bit scabious, *Succisa pratensis*, and teasel, *Dipsacus fullonum*. The tubs were covered with fine netting to exclude parasitoids and were placed outdoors in a sunny and sheltered position. When the larvae reached the penultimate instar, they were transferred to special cages. Each cage was made from a large (55 × 35 cm) propagator. The roof was cut out of the translucent cover and replaced by a tightly fitting, removable lid made from fibreglass/resin. The central area of the lid was cut away and replaced with fine netting. Additional ventilation was provided by large net-covered holes in the lateral walls of the cover. The larvae were now fed on evergreen honeysuckle (*Lonicera*). Sprays of *Lonicera* were placed horizontally upon a thin layer of potting compost covering the floor of the propagator. This was kept fresh using wet 'Oasis' plastic foam wrapped in clingfilm, into which the stems were thrust. At pupation, the larvae ascended to the shady area of the lid and it was easy to remove the newly hardened pupae for cold shocks, using fine forceps. Pairings were easily obtained using hanging flight cages in sunshine inside a well-ventilated greenhouse. However, in contrast to observations on feral *aurinia*, (Thomas & Lewington, 1991), captive females refused to pair until at least 2 days old. The adults were fed on 10% honey solution. Ova were obtained by caging fertile females over growing devil's bit scabious.

Cold-shock technique

Pupae for cold shocks were collected in the early morning, around 2 pm and in the late afternoon. Only those pupae with a fully hardened cuticle were collected, usually when 8–12 hours old. The pupae from each collection were laid in an individually labelled small tray and placed on a much larger tray. This large tray was periodically inserted and withdrawn from a specially modified freezer that could be regulated to operate at $\pm 1^{\circ}C$ down to $-14^{\circ}C$. The pupae were given three 6 hour periods per day at $-6^{\circ}C$ for the first 2–3 days of the pupal stage. The pupae were then held at room temperature until eclosion.

Initial breeding results

All the wild-collected larvae pupated in May 1991. They were given cold shocks. A single aberration intermediate to ab. *sebaldus* appeared but a pairing was not obtained. However, a number of pairings amongst the type adults occurred and a number of fertile egg masses were laid. During February–April 1992, cohorts of post-hibernation larvae were successively brought into a warmed greenhouse area and quickly reared under infra-red enhanced lighting. They were reared on evergreen *Lonicera*. Many pupae developed and were given cold shocks. About 20% of the pupae produced aberrations, all within a morphocline leading to extreme ab. *sebaldus* (see Bailey, 1993, 1994). This sensitivity was confined to the first two cohorts. Succeeding cohorts exhibited a steadily decreasing percentage of aberrations. No aberrations appeared in the final cohorts in April. It would appear that larvae developing over a longer period under natural conditions acquire an increasing immunity to pattern modifications. Possibly this is due to a longer exposure to ultraviolet light (u.v.). The author has found that other species seem to acquire immunity if the larvae are reared under u.v. enhanced lighting. A female with a wing pattern intermediate to ab. *sebaldus* was paired with a normal male and the resulting F_1 stock reared in isolation. In May 1993, this particular stock was reared under natural conditions the pupae were not given cold shocks. All the F_1 adults had normal coloration and pattern (although the geometry of the wing pattern enclosing the normal colours distorts with the expression of ab. *sebaldus*). Five F_1 stock pairings were obtained, yielding in due course a large number of F_2 larvae. During February–March 1994, the post hibernation F_2 larvae were reared all together under a 12 hour photoperiod and in a 19.5 C ambient temperature and the same foodplants. All the developing larvae had normal coloration. As the pupae formed, they were given various cold-shock regimes.

Appearance of a new aberration

Towards the end of the pupations, a very young pupa, only a few minutes old, was noticed having an unusual colour, being very pale and with a slight greenish tinge. This subsequently developed into a most unusual colour form. It had a pure white background and with starkly contrasting black markings in the usual places (Plate I Fig. 4). The normal yellow markings were replaced by blackish maroon. In all, eighteen similar pupae developed. Some of these were found lying on the floor of the breeding cage and these subsequently died. After the appearance of the first aberrant pupa, no further cold shocks were performed on the remaining F_2 stock. Prior to eclosion, the aberrant pupae became extremely dark in colour and it was difficult to see the wing pattern through the pupal cuticle. The adults emerging from these aberrant pupae were quite different from the type form. There were no intermediates and both sexes were equally affected and constant in coloration. Aberrant adults only emerged from aberrant pupae, the two conditions being linked.

Description of the new aberration, ab. *atratus*.

(Plate I Figs 5–8)

The antennal flagella are black, annulate pale grey and with the clubs narrowly marked with pale grey at the tips. All the normal antennal orange is replaced with blackish maroon. The eyes are grey. The frons and labial palpi have the normal orange scales replaced by dark maroon ones. The dorsal thorax and lateral abdomen

have the normal orange scaling replaced by dark maroon hair scales. The ventral thorax and abdomen are dark grey. The legs and genitalia are blackish maroon. The male upperside fore and hindwings have all the dark pattern (the geometry being normal), replaced by smoky greyish black. The cream ground is replaced by purple buff. The orange-red ground colour is replaced by dark red ochre. The cilia are greyish buff. The male underside hindwing has the cream subterminal band replaced by smoky greenish blue. The normal orange-red ground colour in the basal area and the postmedian band is replaced by dark reddish ochre. The remaining underside cream markings are replaced by smoky buff. The underside forewings are similar but with an accentuated smoky purple hue particularly in the central area. The overall coloration is paler. All the aberrant adults were slightly smaller than normal. The post-coital deposit from a male ab. *atratus* left covering the female ostium bursae was of normal colour, as was the meconium. The name derives from *atratus* = sombre, in mourning, Latin. The original ab. *atratus* are in the author's collection.

Behaviour

The first two ab. *atratus*, both males, emerged after the usual pupal duration. All the ab. *atratus* were fairly synchronous and appeared among the slowest developers. The two males were placed in a small flight cage with several unmated type females. The males were weak and with minimal motility. Neither male would mate, therefore all subsequent *atratus* males were set. In all, six *atratus* females emerged. These were much stronger and three pairings with type F_2 stock males were obtained. From these pairings, fertile egg masses were obtained which were smaller than average. From these egg masses, a large number of larvae resulted with a minimal number of failures. The pairing females looked remarkable against the normal-coloured males (Plate 1, Figs 7, 8) reminiscent of the pairing of *Argynnis paphia* f. *valezina* Esp. The *atratus* females were more sluggish than normal, hiding away under leaves in bright sunshine or resting with their wings parallel to the incident sunlight, presumably to avoid overheating.

Breeding results from the ab. *atratus* females

All the larvae from the ab. *atratus* females, called F_3, were reared *en masse* in the normal way but kept in strict isolation. On 1.ii.95, a cohort of 60 post-hibernation larvae, called f_3, was extracted and reared rapidly in warmth and u.v. enhanced lighting. By 26.ii.95, the first adults emerged. All the f_3 adults were normal. A number of pairings were obtained followed by fertile egg masses, to produce f_4 larvae. These were closely observed but no abnormalities were noted. On 25.iii.95, the first larvae commenced feeding and by 21.iv.95, the first larvae entered diapause. On 15.v.95, all the larvae were in diapause. They were placed amongst sterilized dead Beech leaves (*Fagus sylvatica*), in two separate containers, strains f4a and f4b, and held in a freezer at -2.5°C. On 15.viii.95, the first container holding f4a larvae was removed and placed amongst growing foodplant. By 19.viii.95, many larvae were feeding. From this stock, one ab. *atratus* appeared at the rate of 0.98%. However, some f4a larvae had remained in diapause and these were returned to the freezer until 15.x.95, when all the remaining larvae, being f4a and f4b, were reared as post-hibernation stock. From this stock, ab. *atratus* appeared at the rate of 11% with characters identical to the aberrants in the original 1994 F_2 stock. The reappearance of ab. *atratus* in such reduced numbers after further inbreeding suggests the action of a recessive gene(s) with a weakening effect, and with a lengthened diapause

requirement. After the October 1995 breeding programme, a few larvae remained in diapause. These were kept in a cold area until February 1996.

Returning to March 1995, the remaining F_3 larvae, from the 1994 ab. *atratus* females, were reared under natural conditions. All the adults were normal again. These were paired amongst themselves to produce a further generation, F_4, and in February–March 1996, the post-hibernation larvae were merged with the remaining diapausing larvae, f4a and f4b from October 1995. Ab. *atratus* appeared at the rate of 9%. Three *atratus* females were paired with stock from a separate Devonshire colony to invigorate the strain which was showing signs of weakening. A single pairing was obtained between a male and female ab. *atratus* but the resultant egg mass failed to hatch. Assuming the ab. *atratus* was recessive, all type adults, F_3, in May 1995 would have been heterozygous, so several males were paired with females of the Spanish race *E. aurinia beckeri* H.-S. The F_1 hybrids which were vigorous emerged in May 1996 and appeared to be intermediate between the two types but there was no trace of *atratus* characters. Presumably, *atratus* gene(s) are recessive in the Spanish race. A large number of F_2 hybrid larvae are being raised. A curious aberration appeared amongst the 1996 adults. A type female presented with streaks of *atratus* colouring in the distal compartment of the left underside hindwing. Perhaps this was due to the loss or damage to the corresponding dominant gene(s) in a primordial cell in the appropriate imaginal disc of an otherwise heterozygous specimen. Breeding stock has been obtained from this female for further research.

Conclusion

Ab. *atratus* is most probably genetic in origin and the occurrence of separate inherited aberrant characters in more than one stage of the same individual is a very rare condition in the rhopalocera. The author hopes to extend this breeding programme by hybridizing *atratus* with other races of *E. aurinia*. The origin of this genetic form is obscure. Perhaps heterozygous *atratus* was in the 1991 wild-collected larvae. If so, there would be heavy selective pressure against a homozygote ever breeding. Alternatively, perhaps a mutation was induced by cold shock after the culture was established. It would be remarkable, if proven, because the altered colours of both stages, as seen by man, and the extended diapause time would seem better suited to an alpine or boreal environment.

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References

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Bailey, K. E. J. 1994. Annual Exhibition Report 1993. *Br. J. Ent. Nat. Hist.*, **7**: 145–6, Plate 1 Fig. 5 (photographs of cold-shock-induced ab. *sebaldus*).

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